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(11)

**EP 1 118 329 A1**

(12)

**EUROPEAN PATENT APPLICATION**  
published in accordance with Art. 158(3) EPC

(43) Date of publication:  
25.07.2001 Bulletin 2001/30

(51) Int Cl.<sup>7</sup>: **A61K 38/02, A61P 27/02**

(21) Application number: **99944828.5**

(86) International application number:  
**PCT/JP99/05239**

(22) Date of filing: **24.09.1999**

(87) International publication number:  
**WO 00/18422 (06.04.2000 Gazette 2000/14)**

(84) Designated Contracting States:  
**AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU  
MC NL PT SE**

(30) Priority: **28.09.1998 JP 27333298**

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(54) **LACRIMAL SECRETION PROMOTERS OR EYE DROPS FOR TREATING  
KERATOCONJUNCTIVAL FAILURE CONTAINING AS THE ACTIVE INGREDIENT  
NATRIURETIC PEPTIDES**

(57) An object of the present invention is to find new effects of natriuretic peptides in an ophthalmological field. The present invention provides eyedrops for promoting lacrimal secretion or for treating keratoconjunctival disorders containing the natriuretic peptide as ac-

tive ingredient. The natriuretic peptides are atrial natriuretic peptides (ANP), brain natriuretic peptides (BNP) and C-type natriuretic peptides (CNP). Typical examples of the keratoconjunctival disorder are dry eye, corneal erosion and corneal ulcer

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**Description**

## Technical Field

- 5 **[0001]** The present invention relates to eyedrops for promoting lacrimal secretion or treating keratoconjunctival disorders containing a natriuretic peptide as an active ingredient.

## Background Art

- 10 **[0002]** A lacrimal fluid, which has a mechanism to retain wettability on living bodies, covers cornea and conjunctiva (keratoconjunctiva), retains wettability and prevents them from drying. The lacrimal fluid works as a lubricant protecting the keratoconjunctiva from stimulation by blinking and contributes to retaining smoothness of the corneal surface. The lacrimal fluid has bacteriostasis, prevents infection from bacteria, fungus, virus and the like, supplies oxygen and a variety of nutrition to the cornea and removes a carbon dioxide gas and metabolites. When the keratoconjunctiva is  
15 disordered, the lacrimal fluid plays a role to dilute and remove disordering stimuli, and works to carry liquid components such as epidermal growth factors participating in wound healing and hematocyte components such as fibronectin to disordered sites. That is, the lacrimal fluid participates in adjusting wound healing as well as retaining keratoconjunctival epithelial cells. Thus, it is known that the lacrimal fluid, though its amount is very small, adjusts a physiological condition of the keratoconjunctiva, and thereby maintaining transparency and homeostasis of the cornea (Journal of the Eye,  
20 11, 1179-1185 (1994)).

- [0003]** Known methods of treating keratoconjunctival disorders such as dry eye (keratoconjunctivitis sicca and the like) are a method of supplying lacrimal fluid components with artificial tears, a method of retaining a lacrimal fluid remaining on the keratoconjunctiva surface with a viscoelastic substance to lead to treating the keratoconjunctiva and the like. Since the lacrimal fluid exhibits the above-mentioned effect of curing the keratoconjunctival disorders, it is  
25 expected that finding compounds acting on a lacrimal gland function directly and promoting lacrimal secretion is useful for curing corneal erosion, corneal ulcer and the like having keratoconjunctival epithelial disorders such as dry eye.

- [0004]** Peptides belonging to natriuretic peptides are widely distributed in mammal, birds, amphibians and fish and are classified into three groups, namely atrial natriuretic peptides (ANP), brain natriuretic peptides (BNP) and C-type natriuretic peptides (CNP), according to structure. Known atrial natriuretic peptides (ANP) are  $\alpha$ -ANP consisting of 28  
30 amino acids,  $\alpha$ -ANP [4-28] consisting of 4th to 28th amino acids of  $\alpha$ -ANP,  $\alpha$ -ANP [5-28] consisting of 5th to 28th amino acids of  $\alpha$ -ANP,  $\beta$ -ANP having an antiparallel dimer structure of  $\alpha$ -ANP, high-molecular type  $\gamma$ -ANP having molecular weight of 13,000 formed by cutting out a signal peptide from an ANP precursor and the like. Known brain natriuretic peptides (BNP) are BNP-26 consisting of 26 amino acids, BNP-32 consisting of 32 amino acids, BNP-45 consisting of 45 amino acids, high-molecular type  $\gamma$ -BNP having molecular weight of about 13,000 formed by cutting out a signal  
35 peptide from a BNP precursor and the like. Known C-type natriuretic peptides (CNP) are CNP-22 consisting of 22 amino acids, CNP-53 consisting of 53 amino acids extending to the N-terminal and the like. These natriuretic peptides act on kidneys, adrenal glands and vascular walls and play an important role in adjusting electrolytes in a general body fluid and blood pressure ("Peptides Adjusting Circulation and Related Diseases", p. 14-25, Youdosha, 1992).

- [0005]**  $\alpha$ -ANP has a vasodilatory action and a diuretic action and is used as a therapeutic agent for cardiovascular diseases such as heart failure (Jpn. Pharmacol. Ther., 23, 949-952 (1995)).

- [0006]** In an ophthalmological field, it was reported that  $\alpha$ -ANP exhibits an effect of lowering intraocular pressure (Curr. Eye Res., 6, 1189-1196 (1987)). However, other effects of  $\alpha$ -ANP have scarcely been studied, and there have been no literature which reports effects of the natriuretic peptides on lacrimal gland and the keratoconjunctival disorders by instilling them.

- 45 **[0007]** Few application studies of the natriuretic peptides to the ophthalmological field have been done except for the study on the effect of lowering intraocular pressure. It is a very interesting subject to study new effects of the natriuretic peptides in the ophthalmological field.

## Disclosure of the Invention

- 50 **[0008]** Studying precisely in order to find new effects of natriuretic peptides in an ophthalmological field, the present inventors found that the natriuretic peptides exhibit effects of promoting lacrimal secretion and are useful as therapeutic agents for keratoconjunctival disorders.

- [0009]** The present invention relates to eyedrops for promoting the lacrimal secretion and treating the keratoconjunctival disorders containing the natriuretic peptide as an active ingredient.

- 55 **[0010]** The natriuretic peptides are atrial natriuretic peptides (ANP), brain natriuretic peptides (BNP) and C-type natriuretic peptides (CNP) in the present invention. ANP, BNP and CNP having different structures are known, and the natriuretic peptides of the present invention include all of them.

[0011] The natriuretic peptides are useful drugs as therapeutic agents for cardiovascular diseases, but few effects have been reported other than their effects of lowering intraocular pressure in an ophthalmological field.

[0012] Studying application of the natriuretic peptides to the ophthalmological field, the present inventors found that when the natriuretic peptides are instilled into rabbits, the natriuretic peptides exhibit excellent effects of promoting the lacrimal secretion. Details will be described in the part of "Pharmacological Test". Since the lacrimal fluid exhibits the effect of curing the keratoconjunctival disorders as described in detail in the part of "Background Art", the present drugs are expected to be useful as the therapeutic agents for the keratoconjunctival disorders. Typical examples of the keratoconjunctival disorder are dry eye, corneal erosion and corneal ulcer.

[0013] The eyedrops of the present invention can be prepared by dissolving the natriuretic peptide in a general ophthalmic vehicle in using the eyedrops. The eyedrops can be formulated by adding optionally a suitable amount of an isotonic agent such as sodium chloride or concentrated glycerin, a buffer such as sodium phosphate or sodium acetate, a surfactant such as polyoxyethylene sorbitan monooleate, polyoxyl 40 stearate or polyoxyethylene hydrogenated castor oil, a stabilizer such as sodium citrate or disodium edetate, a preservative such as benzalkonium chloride or paraben or the like. pH can be in the range acceptable to ophthalmic preparations and is preferably in the range of 4 to 8.

[0014] A concentration of the active ingredient in the eyedrops is 0.001 to 1% (W/V), preferably 0.005 to 0.5% (W/V), more preferably 0.05 to 0.5% (W/V). The eyedrops are administered by instilling one to several times per day.

#### Best Mode for Carrying out the Invention

[0015] Formulation Example and Pharmacological Test are shown below as Examples.

#### 1. Formulation Example

[0016] Typical formulation is shown below.

##### Formulation 1 (Preparation of 0.1% eyedrops)

[0017] A natriuretic peptide (100 mg) was dissolved in physiological saline (100 ml) to prepare 0.1% eyedrops.

[0018] Further, varying the amount of the natriuretic peptide to be added, natriuretic peptide eyedrops having concentrations of 0.001%, 0.005%, 0.01%, 0.05%, 0.5% and 1.0% (W/V) were also prepared.

#### 2. Pharmacological Test

[0019] The Schirmer test paper method, which is used for measurement of an amount of a human lacrimal fluid, is one method of measuring a change in an amount of a lacrimal fluid of a normal animal in instilling a drug. In the present invention, an amount of a lacrimal fluid in instilling the natriuretic peptide was measured by using the Schirmer test paper method, and an effect of the natriuretic peptide on a rate of lacrimal secretion was studied.

#### Experimental method

##### Laboratory animal

[0020] Male Japanese white rabbits, body weight; 1.8 to 2.2 kg, were used for the experiment.

##### Preparation of solution of test drug

[0021] The natriuretic peptide (0.56 mg) was dissolved in sterile purified water to prepare a 0.1% solution just before using. This solution is referred to as a solution of a test drug.

##### Method of administering drug

[0022] The solution of the test drug (50  $\mu$ l) was instilled into a left eye once. In order to study an effect of a vehicle (purified water) on the lacrimal secretion, the vehicle alone (50  $\mu$ l) was instilled into a right eye once.

##### Method of measurement

[0023] Amounts of the lacrimal fluid were measured using Schirmer test paper with respect to the eye to which the

drug was administered and the eye to which no drug was administered before administering the drug and after a prescribed period of time from the instillation. One end of the Schirmer test paper was folded, and the folded end was inserted into a site of one third of palpebrae inferior toward a temporal side of the rabbit. One minute later, length of a wet portion (Schirmer value, mm) of the test paper was measured from the fold.

[0024] Three minutes before measuring the amount of the lacrimal fluid, a 0.4% ophthalmic solution of oxybuprocaine hydrochloride (10  $\mu$ l), which is a local anesthetic, was instilled into both eyes once.

#### Results

[0025] The action of the solution of the test drug on the rate of the lacrimal secretion at a time of T hours after the instillation is represented by the increment of the lacrimal fluid (mm) determined by the following equation.

[0026] Increment of lacrimal fluid (mm)

$$=[SM(D-T)-SM(D-0)]-[SM(V-T)-SM(V-0)]$$

SM(D-T): Schirmer value in the eye to which the drug was administered (right eye) T hours after administering the drug

SM(D-0): Schirmer value in the eye to which the drug was administered (right eye) before administering the drug

SM(V-T): Schirmer value in the eye to which the vehicle was administered (left eye) T hours after administering the vehicle

SM(V-0): Schirmer value in the eye to which the vehicle was administered (left eye) before administering the vehicle

[0027] As examples of test results, Table 1 shows increments of the lacrimal fluid (mm) one hour after instilling solutions of test drugs (rat  $\alpha$ -ANP, rat BNP-32 and human CNP-22; all of them were purchased from Peptide Research Institute).

Table 1

Measuring time	Increment of lacrimal fluid (mm)		
	Rat $\alpha$ -ANP	Rat BNP-32	Human CNP-22
One hour after administering drug	+1.92	+2.08	+1.33

[0028] The values in the table are respective averages of six samples per group.

[0029] As apparent from Table 1, the test drugs (rat  $\alpha$ -ANP, rat BNP-32 and human CNP-22) exhibit excellent effects of promoting the lacrimal secretion.

#### Industrial Applicability

[0030] The present invention can provide eyedrops containing a natriuretic peptide which exhibit an excellent effect of promoting lacrimal secretion and are useful as lacrimal secretion promoters and as therapeutic agents for keratoconjunctival disorders.

#### Claims

1. Eyedrops for promoting lacrimal secretion containing a natriuretic peptide as an active ingredient.
2. Eyedrops for treating a keratoconjunctival disorder containing a natriuretic peptide as an active ingredient.
3. The eyedrops for treating the keratoconjunctival disorder as claimed in claim 2, wherein the keratoconjunctival disorder is at least one selected from dry eye, corneal erosion and corneal ulcer.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP99/05239

A. CLASSIFICATION OF SUBJECT MATTER  
Int.Cl.<sup>7</sup> A61K38/02, A61P27/02

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
Int.Cl.<sup>7</sup> A61K38/02, A61P27/02

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
CAPLUS (STN), REGISTRY (STN), WPI (DIALOG), MEDLINE (STN)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LANGE, Winfried, et al., "Localization of Atrial Natriuretic Peptide/Cardiodialatin (ANP/CDD) - Immunoreactivity in the Lacrimal Gland of the Domestic Pig", Exp. Eye Res., No. 50, 1990, pp. 313-316, Abstract, Discussion	1-3
Y	JP, 10-218792, A (SANTEN PHARMACEUTICAL CO., LTD.), 18 August, 1998 (18.08.98), Claims; Par. No. [0002] (Family: none)	1-3
Y	JP, 10-236972, A (Mitsubishi Chemical Corporation), 08 September, 1998 (08.09.98) Claims; Par. No. [0002] (Family: none)	1-3
Y	EP, 385476, A1 (Daiichi Pure Chemicals Co., Ltd.), 01 March, 1990 (01.03.90), Full text & JP, 2-231082, A & JP, 2-237999, A	1-3

☒ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

\* Special categories of cited documents:  
 "A" document defining the general state of the art which is not considered to be of particular relevance  
 "E" earlier document but published on or after the international filing date  
 "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)  
 "O" document referring to an oral disclosure, use, exhibition or other means  
 "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention  
 "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone  
 "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art  
 "&" document member of the same patent family

Date of the actual completion of the international search  
17 December, 1999 (17.12.99)

Date of mailing of the international search report  
28 December, 1999 (28.12.99)

Name and mailing address of the ISA/  
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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP99/05239

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP, 466174, A1 (Hisayuki MATSUO, ), 12 July, 1991 (12.07.91), Full text & JP, 4-74198, A & US, 5340920, A	1-3
A	US, 4748232, A (Suntory Limited), 31 May, 1988 (31.05.88) & JP, 60-136596, A & EP, 147193, A & AU, 8437133, A	1-3

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